European Journal of Pharmaceutics and Biopharmaceutics 77 (2011) 424–429

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Ultrasound active nanoscaled lipid formulations for thrombus lysis

Andreas Becker [a,*], Elena Marxer [b], Jana Brüßler [b], Anne Sophia Hoormann [a], Daniela Kuhnt [a], Udo Bakowsky [b], Christopher Nimsky [a]

[a] University of Marburg, Department of Neurosurgery, Marburg, Germany
[b] University of Marburg, Department of Pharmaceutical Technology and Biopharmaceutics, Marburg, Germany

ARTICLE INFO

Article history:
Available online 17 December 2010

Keywords:
Ultrasonography
Nanomedicine
Thrombolysis
Contrast agent
Microbubbles

ABSTRACT

In the present study, we investigated the sonothrombolytic effect of new nanoscaled lipid formulations in human blood clots, using diagnostic ultrasound. Human blood clots of 1 ml were incubated with 1 µl of the different lipid dispersions DPPC/CH, DPPC/PEG40S, DSPC/PEG40S and the commercially available ultrasound contrast agent SonoVue®. Clots were stored for 3 days at 5 °C to obtain maximal clot retraction and lytic resistance. Each clot weight was determined before and after continuous insonation for 1 h of insonation at 1.4 MHz. The pressure in the insonation chamber was 80 mm Hg to mimic middle arterial blood pressure. There were no significant differences in thrombus weight before insonation. All nanoscaled formulations and SonoVue® were able to reduce thrombus weight compared to the weight loss of clots that were not insonated but kept under pressure for one hour ($p < 0.001$). We found a highly significant weight reduction with DSPC/PEG40S compared to SonoVue® ($p = 0.007$).

Nanoscaled DSPC/PEG40S dispersion could be a promising formulation in ultrasound enhanced thrombolysis even without thrombolytic drugs. Stable cavitation is a crucial parameter in fragmentation of thrombus architecture. Further studies of physicochemical properties of DSPC/PEG40S are necessary to corroborate our hypothesis.

1. Introduction

The ischemic stroke is one of the most frequent causes of death (after cardiovascular diseases and malignomas) in the federal republic of Germany with an incidence of approximately 160–240 of 100,000. The lethality according to the Erlangen Stroke register is high with 19.4% within the first 28 days after incident, 28.5% within the first three months after incident and even 37.3% within 12 months after incident. The ischemic stroke is one of the most common diseases in neurological daily routine. Over the last years, the mortality-rates decreased significantly due to the establishment of thrombolysis.

The use of recombinant tissue plasminogen activator (rtPA) is the only established therapy in acute vessel occlusion. Evidence from in vitro experiments indicated that application of ultrasound (US) is a promising approach for thrombolysis and therefore recanalisation of occluded vessels, e.g. in acute ischemic stroke [1,2]. Ultrasound together with commercially available microbubbles (MB) as an ultrasound contrast agent (UCA) can significantly enhance the effect of sonothrombolysis and can be a new approach in the treatment of acute vessel occlusion as shown in numerous studies. Although the mechanism of MB enhanced thrombolysis is not completely understood, it probably involves both stable and inertial cavitation.

Cavitation as a mechanical property of US is the ability to create short life bubbles from gas dissolved in a liquid medium. Probably, cavitation is not achieved with US at low intensity as used for clinical diagnostic. However, adding MB may lower the threshold for cavitation. The specific acoustic properties of the MB result in two types of cavitation during insonation. Stable cavitation results in oscillation and translation of the MB, generating subharmonics and microstreaming. This will cause local mechanical stress to the adjacent thrombus, the clot surface will erode, and the penetration of rtPA is now possible [3]. Increasing acoustic power leads to bubble collaps and breaks them up into smaller bubbles with wall velocities of hundreds of meters per second (inertial cavitation) [4]. Inertial cavitation produces microjets that are also effective in eroding the thrombus [5]. Recent data suggest that stable cavitation is more effective than inertial cavitation [6]. The mechanical index (MI) is an estimation of the maximum amplitude of the pressure pulse in tissue and responsible for cavitation:

$$\mathrm{MI} = \frac{P_{\mathrm{neg}}}{\sqrt{f_0}}$$

with P_{neg} as the negative acoustic pressure in MPa and the fundamental frequency f_0 in MHz. The MI of the ultrasound beam is used

* Corresponding author. University of Marburg, Department of Neurosurgery, Baldingerstrasse 1, 35033 Marburg, Germany. Tel.: +49 6421 5863844; fax: +49 6421 5866415.
E-mail address: andreas.becker@staff.uni-marburg.de (A. Becker).

0939-6411/$ - see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2010.12.003

to modulate the output signature of UCA to stimulate different MB responses. At a low MI (<0.2), MB undergo compression and rarefaction equally in amplitude and transmitted frequency (the fundamental frequency) resulting in linear pulsatility. At higher MI (0.2–0.5), rarefaction is greater than compression, and MB-specific nonlinear oscillation occurs at harmonics (second harmonic at twice the fundamental), subharmonics (half of fundamental) or ultraharmonics (1.5–2.5 times of fundamental). At high amplitudes (MI > 0.6), the expansion phase is followed by a violent collapse.

To improve diagnostic ultrasound imaging techniques and to develop new therapeutic applications, MB have been modeled. The nonlinear oscillating behavior of a spherical symmetric bubble has been described by the models based on the Rayleigh–Plesset equation [7] and was modified by de Jong and Hoff by adding shell stiffness and friction terms [8]

$$f_0 = \frac{1}{2\pi r_0}\sqrt{\frac{3\gamma}{p_0}\left(P_0 + \frac{2\sigma}{r_0}\right) - \frac{2\sigma}{p_0 r_0} + \frac{8\pi r_0^2 S_p}{m}}$$

where γ denotes the adiabatic ideal gas constant, r_0 is the bubble radius and $m = 4\pi r_0^2 p_0$. P_0 and p_0 are the ambient fluid pressure and density, respectively. σ is the surface tension coefficient, and S_p is the shell elasticity parameter. For a given medium, the resonance frequency of a microbubble is inversely proportional to the bubble radius. Church derived a general theoretical model for a bubble whose surface is occupied by molecules that behave as a continuous, damped and elastic solid [9]. This model has been modified many times to predict dynamic behavior of UCA. Most of these models are referred to as zero-thickness encapsulation models, assuming a very thin bubble shell. Doinikov and Dayton generalized Church's theory allowing for translational motion and radiation losses due to the compressibility of the surrounding liquid [10].

The aim of our study was to compare the thrombolytic efficacy of medical US combined with the commercially available UCA SonoVue® and new US active nanoscaled lipid dispersions DPPC/CH, DSPC/PEG40S and DPPC/PEG40S.

2. Methods

2.1. Materials

For the flow model, bovine serum albumin and TRIS hydrochloride were purchased from Sigma–Aldrich Chemie GmbH, Steinheim, Germany. Agar and glycerine were purchased from Carl-Roth GmbH & Co. KG, Karlsruhe, Germany, and natriumacide was purchased from Merck-Chemicals KGaA, Darmstadt, Germany. Antibody against human fibrin IgG1 was purchased from American Diagnostica Inc., Stamfort, USA and the detection kit from Dako Austria (EnVison™ Detection System peroxidase/DAB+, Rabbit/Mouse, Dako No. K5007).

The lipids 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) were purchased from Lipoid GmbH (Ludwigshafen, Germany), cholesterol (CH) and polyethylene glycol (40) stearate (PEG40S) from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). Stock solutions of the lipids (approx. 10 mg/ml) were prepared in a mixture of chloroform:methanol (2:1, v:v) and stored at 4 °C. Chloroform (HPLC-grade) and methanol (HPLC-grade) were purchased from Fisher Scientific (Loughborough, UK).

SonoVue® is a commercially available UCA from Bracco Diagnostics Inc., Milano, Italy. The microbubble dispersion of SonoVue® consisting of a lipid mixture of polyethylene glycol 4000, DSPC, DPPG (1,2-dipalmitoyl-sn-glycero-3-phosphoglycerol) and palmitic acid with sulfurhexafluoride was prepared according to the manufacturer's instructions.

2.2. Preparation of liposomes

The lipid dispersions, DPPC:CH 70:30 (mol:mol, DPPC/CH), DPPC:PEG40S 98:2 (mol:mol, DPPC/PEG40S) and DSPC:PEG40S 98:2 (mol:mol, DSPC/PEG40S) were prepared using the thin film hydration method according to [11,12]. Briefly, from a stock solution (chloroform:methanol, 2:1), the lipids were mixed in a round bottom flask to a total amount of 10 mg lipid. The lipid mixture was dried to a thin film in a rotary evaporator (Heidolph Laborota 4000 efficient, Heidolph Instruments, Schwabach, Germany) under vacuum at 40 °C. The resulting film was rehydrated with 1 ml of phosphate-buffered saline (PBS) pH 7.4 (0.15 mol/l). After vigorous shaking, the lipid dispersions were sonicated in a bath-type sonicator (Bandelin Sonorex RK 100H, Bandelin Electronics, Berlin, Germany, maximal energy) for 20 s at 55 °C (DPPC/CH, DPPC/PEG40S) or at 65 °C (DSPC/PEG40S). Finally, after incubation for 60 min at the above mentioned temperatures, the dispersions were subsequently sonicated again for 2 min. The lipid formulations were stored at 4 °C.

2.3. Physicochemical characterisations

The physicochemical properties of the lipid formulations were investigated using Photon Correlation Spectroscopy (PCS), Laser Doppler Velocimetry (LDV) and Atomic Force Microscopy (AFM) according to [13,14].

2.4. Insonation model

A closed-loop flow system was used, filled with 240 ml of Tris-albumin buffer (0.15 mol/l NaCl, 0.002 mol/l Tris–HCl, 0.1% bovine serum albumin, pH = 7.4). The Tris-albumin buffer reservoir was placed in a waterbath with a constant temperature of 37 °C. A roller pump created a pulsatile flow rate of 50 ml/min. The insonation chamber was designed using a 2000 ml polypropylene beaker. A silicon tube (C-Flex™, Cole-Parmer Inc., Illinois, USA) with an inner diameter of 3 mm was inserted in the beaker with an ascending angle of 60°. The beaker was filled with a solution of 3% agar in 85.5% aqua bidest, 11% glycerine and 0.5% natriumacide as a biocide and allowed to gel. The insonation chamber was stored at 5 °C and was heated up to 37 °C before each experiment. An arterial line for pressure measurement was inserted into the C-Flex™ tube as shown in Fig. 1. A flush bag with 500 ml of 0.9% sodium chloride solution was attached to the transducer via tubing. The flush bag was kept under pressure by placing it in a sleeve that, when inflated to 300 millimetres of mercury (mm Hg), enables a continuous flow of 3–5 ml/h and so prevents Tris-albumin buffer from tracking up the tubing. A flow resistor distal of the i.a. line allows reducing the lumen of the tube, so that a mean system pressure of 80 mm Hg was reached.

The insonation chamber fulfills the requirements of the IEC 61685 standards for doppler and duplex devices with an acoustic velocity of 1541 m s^{-1} (±3) and an acoustic impedance of 1.62 × 10^6 kg m^{-2} s^{-1} (±0.01) [15].

2.5. Ultrasound device

Ultrasound was emitted using a commercial medical diagnostic ultrasound device (Siemens, Sonoline Elegra, Erlangen, Germany). A phased array transducer with a fundamental frequency of 2.5 MHz was mounted on the agar surface of the ultrasound chamber. The transducer provides an axial resolution of 0.7 mm. The lateral resolution depends on the width of ultrasonic beam and can be approximated to be 3 mm. The penetration depth was set to 10 cm, and dynamic range was 45 dB. The scanning density was set to 4. The MI was 0.4 to achieve nonlinear oscillation of

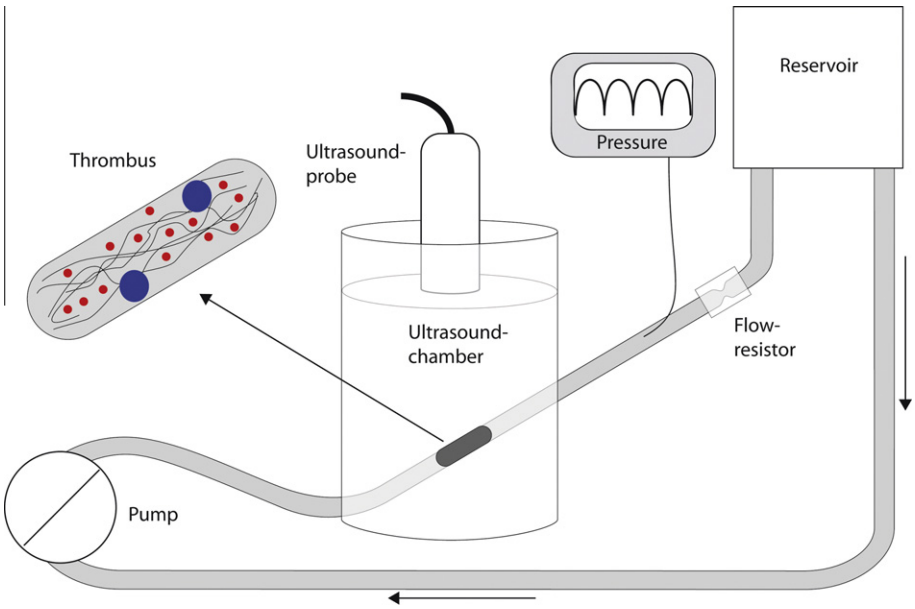

Fig. 1. Ultrasound flow model. Thrombus was placed into the insonation chamber. Red dots in the clot illustrate nanosized lipid formulations, blue dots illustrate SonoVue® within the fibrin mesh. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the MB and therefore harmonics of the fundamental frequency. The overall gain was set to an optimal value to get uniform visibility. The cranial thermal index was 1.3.

2.6. Preparation of blood clots

Human blood was drawn from one healthy volunteer by sterile venipuncture. Seven samples of 1 ml each were placed in 2 ml Eppendorf tubes and mixed with 1 µl SonoVue®, 1 µl DPPC/CH, 1 µl DSPC/PEG40S, 1 µl DPPC/PEG40S or without an UCA resulting in a total number of 35 samples. The clots were incubated for 2 h at 37 °C and subsequently refrigerated at 4–5 °C for 3 days, ensuring maximal clot retraction, lytic resistance and stability [16,17].

2.7. Thrombolytic experiments

Before insonation, the clot was removed from the Eppendorf tube and weighed (Sartorius Analytic Model A 120 S, Germany). Then, the thrombus was inserted into the C-Flex™ tube and carefully aspirated under US control into the insonation chamber. The pressure in the tube was then set to 80 mm Hg to mimic middle arterial blood pressure. Clots were exposed to five treatment regimens, with US (+US) and SonoVue®, +US and DPPC/CH, +US and DSPC/PEG40S and +US and DPPC/PEG40S or without (−US). Each clot was treated for one hour. After treatment, the clots were carefully removed and weighed again.

2.8. Thrombus immunohistochemistry

After paraffin-embedding, 3 µm thick sections were cut and used for immunohistochemical analysis. Immunohistochemistry was performed with monoclonal human fibrin IgG1 antibodies. In a second step, the clots were incubated with Dako EnVision™, Rabbit/Mouse reagent. This reagent is a peroxidase-conjugated polymer, which also carries antibodies to rabbit and mouse immunoglobulins. The reaction is visualised by Dako REALTM DAB + Chromogen.

A single factor analysis of variance (ANOVA) was used to compare the mean values of weight reduction for each blood clot. *p*-Values < 0.05 were considered statistically significant.

3. Results

An ischemic stroke is a medical emergency and can cause permanent neurological disability. A fast recanalisation of the occluded vessels within a 3 h time window is the basis for a possible recovery of the neurological symptoms. Sonothrombolysis using an ultrasound contrast agent is one of the most promising new approaches in the treatment of acute vessel occlusion. The aim of our study was to compare the thrombolytic efficacy of medical US combined with the commercially available UCA SonoVue® and new US active nanoscaled lipid dispersions DPPC/CH, DPPC/PEG40S and DSPC/PEG40S.

3.1. Physicochemical characterisation

The model liposome DPPC/CH showed an average diameter of 223.5 ± 5.4 nm, the new lipid dispersions DPPC/PEG40S and DSPC/PEG40S, 199.8 ± 6.7 and 215.2 ± 2.8 nm, respectively. Whereas the polydispersity index (PdI) of DPPC/CH (0.28) indicates a monodisperse distribution, the PEG40S containing formulations showed higher values of about 0.54 indicating a polydisperse size distribution. The AFM investigations confirmed this data (Fig. 2). The lipid vesicles are spherically shaped. The commercially available ultrasound contrast agent SonoVue® could not be analysed by PCS because of the strong flotation tendency during the measurements; only AFM gave the opportunity to visualise the size and shape (1732 ± 12.6 nm, Fig. 2D) of the microbubbles.

3.2. Thrombolytic experiments

We found no significant differences in thrombus weight before insonation ($p = 0.867$). SonoVue® as well as DPPC/CH, DSPC/PEG40S and DPPC/PEG40S was able to reduce the clot weight after 1 h of insonation. The mean clot weight reduction was 23.1% (±6.8) for SonoVue®, 36.3% (±9.5) for DPPC/CH, 35.4% (±7.6) for DSPC/PEG40S and 32.2% (±10.5) for DPC-PEG-40–stearate. Clots without insonation but under pressure in the chamber showed a weight reduction of 10.2% (±2.9) (Table 1, Fig. 3).

Compared to the −US group, we found highly significant thrombus weight reduction during thrombus treatment with DPPC/CH,

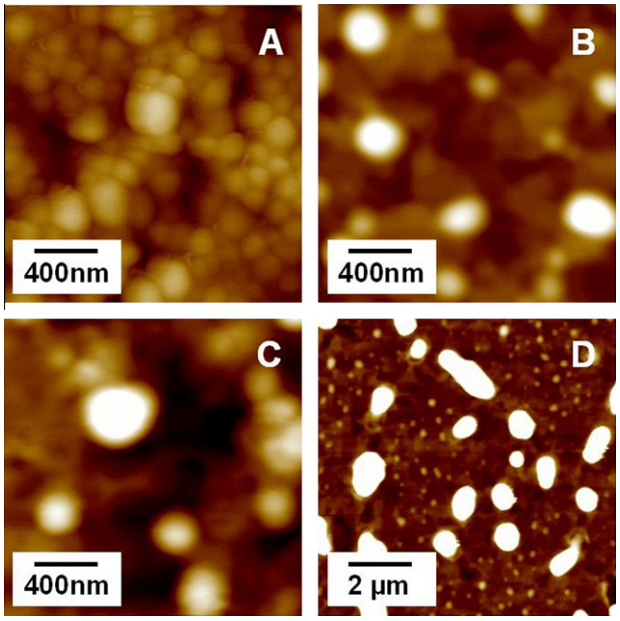

Fig. 2. Atomic force microscopy: (A) SonoVue®, (B) DPPC/CH, (C) DPPC/PEG40S and (D) DSPC/PEG40S. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

DSPC/PEG40S and DPPC/PEG40S ($p < 0.001$). Comparing −US to SonoVue®, the weight reduction was significant ($p = 0.07$), too.

We found no significant differences in weight reduction comparing DPPC/CH and DSPC/PEG40S ($p = 0.57$), DPPC/CH and SonoVue® ($p = 0.23$) and comparing DPPC/PEG40S with DSPC-PEG-4-stearate ($p = 0.5$). We found a significant weight reduction comparing DSPC/PEG40S and SonoVue® ($p = 0.007$).

3.3. Thrombus immunohistochemistry

Immunohistochemical staining was performed for all blood clots after insonation, visualising the fibrin mesh. The native thrombus mesh (before insonation) was compared to those that were preformally treated with one of the nanoscaled lipid formulations or the commercially available SonoVue® and US.

Opposed to a compact fibrin mesh of the untreated thrombus (Fig. 4D), all other mesh formations appear in diminished in density, which correlates to the determined clot weight loss. Although there was no significant reduction in clot weight comparing DPPC/CH or DPPC/PEG40S to SonoVue®, there obviously is a thinner fibrin mesh-structure after application of the nanoscaled lipid formulations (Fig. 4A and B) and US. Despite a subjectively similar mesh-structure after treatment with DSPC/PEG40S, we found a significant clot weight reduction compared to SonoVue®.

4. Discussion

In this experimental study, all new designed nanoscaled lipid formulations were effective in clot weight reduction after 1 h of insonation. These findings are consent with positive results from in vitro and in vivo studies [18–21].

A mechanical property of US is the acoustic cavitation and the ability to create microbubbles of gas dissolved in the surrounding liquids. This cavitation will not be achieved with low MI of diagnostic US. Ultrasound contrast agents act as nuclei for ultrasound induced cavitation, and therefore, less energy is needed for cavitation. When a MB passes through an US-field, size oscillations are generated. With increasing acoustic power, the MB collapses leading to intense localized stresses and microjets (inertial cavitation). Microjets are able to cause mechanical fragmentation of a thrombus [22].

Previous in vitro models of sonothrombolysis showed that various UCAs have different impacts on clot lysis [23]. Increased stability of the MB shell caused higher echo intensity of the thrombus superficial layer and increased weight loss.

Whereas we found no significant weight reduction comparing SonoVue® with DPPC/CH and DPPC/PEG40S, the thrombus weight reduction was significantly higher by using nanoscaled DSPC/PEG40S ($p = 0.007$).

Taking cavitational mechanism of US into account, we assume that the compressibility and stability of SonoVue®, DPPC/PEG40S, and DPPC/CH are smaller than for DSPC/PEG40S. Therefore, stable cavitation and microstreaming may fragment the thrombus architecture and reduces the thrombus weight even without rtPA. This can be demonstrated by the immunohistochemical findings with a thinned out human fibrin mesh (Fig. 4).

The significant loss of thrombus weight in the absence of rtPA is probably related to an improvement of the intrinsic enzymatic fibrinolysis [24].

Unfortunately, the material properties of phospholipids organised in such US active formulations are recently poorly known. Approximate values for shear modulus and viscosity of the shell were obtained by de Jong and Hoff by fitting experimental data of Albunex® [8]. Krasovitski and Kimmel concluded that diffusion of gas out of the MB might cause a loss of shell stability and lead to collapse of MBs [25]. They also state that the stability of the shell influences the critical radius for collapsing. The more stable the shell, the smaller is its critical radius. Softer shells are more stable. Taking rigidity into account, we hypothesised less rigidity in DSPC/PEG40S. Physicochemical properties of a lipid shell have a substantial effect on the MB response during insonation. Borden et al. could show that the acyl chain length of the phospholipid influenced the rate of acoustic dissolution and fragmentation [26]. This effect was first described by Kim et al. [27]. A higher number of methylene groups per hydrocarbon chain increases the attractive intermolecular dispersion forces between constituent lipids in a shell and thus the overall cohesiveness. Increase in cohesiveness results in greater stability for MB experiencing oscillations due to insonification.

Tata and coworkers investigated the relaxation kinetics of membranes of unilamellar and multilamellar vesicles composed of DMPC or DPPC by measuring the ultrasonic absorption and velocity in such dispersions [28]. Using 1.42 and 2.11 MHz ultrasound for DMPC (T_M 23.2 °C) and DPPC (T_M 41.4 °C), respectively, they found that enhanced ultrasonic absorbance occurs at the main

Table 1
Mean values and standard deviation (±SD) of clot weight (in g) before and after insonation, weight loss in g and percent (%). (+US): with insonation, (−US): without insonation, UCA: Ultrasound contrast agent.

	DPPC-CHOL (+US)	DPPC-PEG-40/stearate (+US)	+US DSPC-PEG-40/stearete (+US)	SonoVue® (+US)	No UCA (−US)
Before	0.179 (±0.025)	0.181 (±0.01)	0.178 (±0.008)	0.197 (±0.02)	0.175 (±0.01)
After	0.120 (±0.023)	0.118 (±0.014)	0.123 (±0.013)	0.143 (±0.02)	0.157 (±0.013)
Weight loss (g)	0.059 (±0.011)	0.058 (±0.02)	0.064 (±0.021)	0.041 (±0.011)	0.017 (±0.005)
Weight loss (%)	36.3 (±9.5)	32.2 (±10.5)	35.4 (±7.6)	23.1 (±6.8)	10.2 (±2.9)

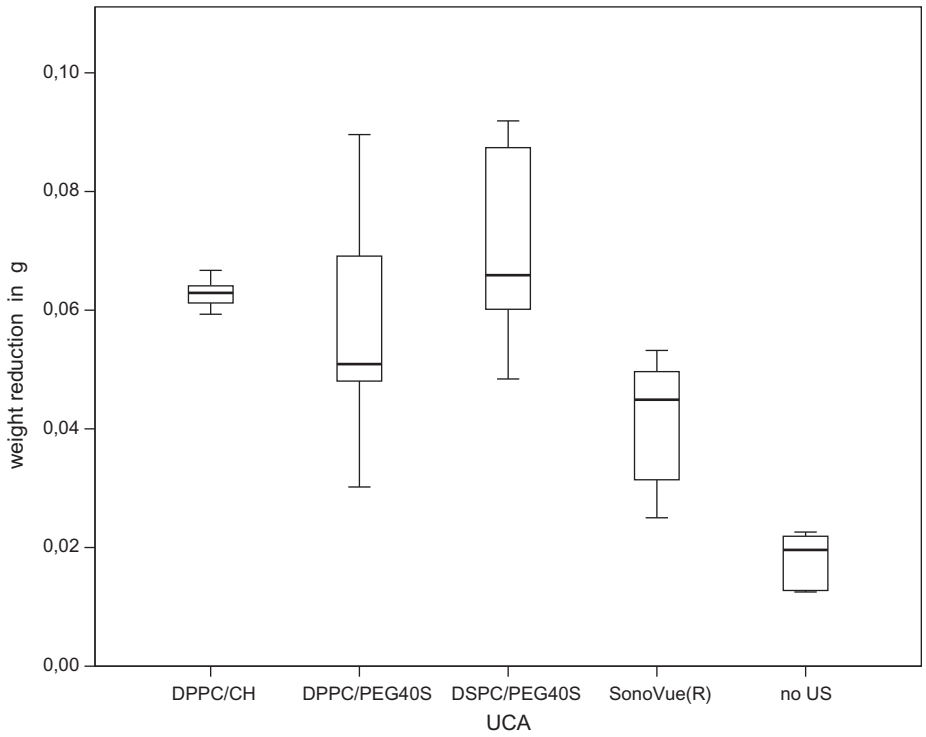

Fig. 3. Clot weight loss. Values are displayed as box plots with the middle line indicating the median, the boxes on both sides of it the interquartile range and the lines extended from the box to the highest and lowest values. Outlier is displayed as a spot.

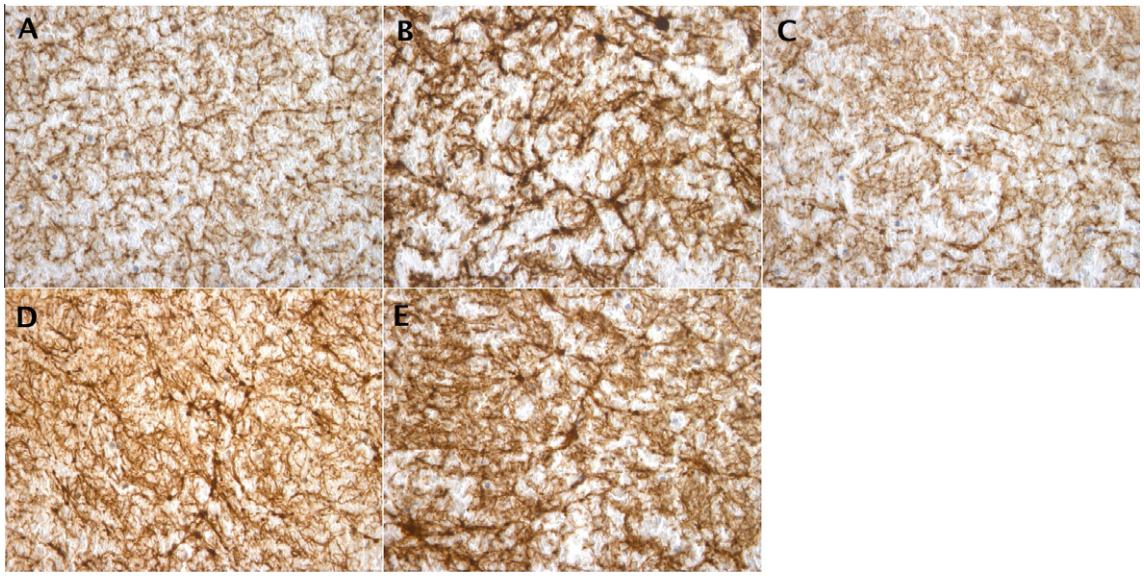

Fig. 4. Representative antifibrin immunohistochemical stainings after one hour of insonation (magnification: 40×): (A) DPPC/CH, (B) DPPC/PEG40S, (C) DSPC/PEG40S, (D) no ultrasound/UCA and (E) SonoVue®. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(solid-ordered (SO) to liquid-disordered) lipid phase transition, while below the phase transition, ultrasound is hardly absorbed by the membrane. This suggests that liposomal drug release, achieved when working below the phase transition temperature (in the SO-phase), may be attributed to mechanical or thermal effects rather than absorbance of ultrasound by the lipid bilayer. A previous study tested the ability to release doxorubicin from Doxil using ultrasound at low or high frequencies (20 kHz, or 1 and 3 MHz). Doxil is a nanoscaled liposomal anti-cancer drug in which doxorubicin is remotely (actively) loaded into 100-nm sterically stabilized liposomes [29].

In summary, the new nanoscaled formulation of DSPC/PEG40S could be a promising UCA for sonothrombolysis.

Further studies of physicochemical properties of DSPC/PEG40S as a thrombolytic enhance drug are necessary to corroborate our hypothesis.

5. Conclusion

New nanoscaled ultrasound active lipid formulations may contribute to thrombus lysis, even without additional application of

fibrinolytic drugs. Especially, DSPC/PEG40S might have the potential to be implemented into the treatment-regime of acute vessel occlusion, e.g. in ischemic stroke.

Acknowledgements

Udo Bakowsky thanks the Deutsche Forschergemeinschaft (DFG) (Forschergruppe 629 "Nanohale" and 495 "Biohybridverbindungen") for financially support.

References

[1] P. Cintas, F. Nguyen, B. Boneu, V. Larrue, Enhancement of enzymatic fibrinolysis with 2-MHz ultrasound and microbubbles, J. Thromb. Haemost. 2 (7) (2004) 1163–1166.
[2] V. Larrue, C. Viguier, Transcranial ultrasound combined with intravenous microbubbles and tissue plasminogen activator for acute ischemic stroke: a randomized controlled study, Stroke (2007) 472 (38).
[3] E.C. Everbach, C.W. Francis, Cavitational mechanisms in ultrasound-accelerated thrombolysis at 1 MHz, Ultrasound Med. Biol. 26 (7) (2000) 1153–1160.
[4] K.E. Morgan, J.S. Allen, P.A. Dayton, J.E. Chomas, A.L. Klibaov, K.W. Ferrara, Experimental and theoretical evaluation of microbubble behavior: effect of transmitted phase and bubble size, IEEE Trans. Ultrason. Ferroelectr. Freq. Control 47 (6) (2000) 1494–1509.
[5] M.W. Miller, D.L. Miller, A.A. Brayman, A review of in vitro bioeffects of inertial ultrasonic cavitation from a mechanistic perspective, Ultrasound. Med. Biol. 22 (9) (1996) 1131–1154.
[6] S. Datta, C.C. Coussios, L.E. McAdory, J. Tan, T. Porter, G. De Courten-Myers, et al., Correlation of cavitation with ultrasound enhancement of thrombolysis, Ultrasound. Med. Biol. 32 (8) (2006) 1257–1267.
[7] C.E. Brennen, Cavitation and Bubble Dynamics, Oxford University Press, 1995.
[8] N. de Jong, L. Hoff, Ultrasound scattering properties of Albunex microspheres, Ultrasonics 31 (3) (1993) 175–181.
[9] C.C. Church, The effects of an elastic solid surface layer on the radial pulsation of gas bubbles, J. Acoust. Soc. Am. (97) (1995) 1510.
[10] A.A. Doinikov, P.A. Dayton, Spatio-temporal dynamics of an encapsulated gas bubble in an ultrasound field, J. Acoust. Soc. Am. 120 (2) (2006) 661–669.
[11] S. Anabousi, M. Laue, C.M. Lehr, U. Bakowsky, C. Ehrhardt, Assessing transferrin modification of liposomes by atomic force microscopy and transmission electron microscopy, Eur. J. Pharm. Biopharm. 60 (2) (2005) 295–303.
[12] H. Bakowsky, T. Richter, C. Kneuer, D. Hoekstra, U. Rothe, G. Bendas, et al., Adhesion characteristics and stability assessment of lectin-modified liposomes for site-specific drug delivery, Biochim. Biophys. Acta 1778 (1) (2008) 242–249.
[13] V. Oberle, U. Bakowsky, I.S. Zuhorn, D. Hoekstra, Lipoplex formation under equilibrium conditions reveals a three-step mechanism, Biophys. J 79 (3) (2000) 1447–1454.
[14] C. Kneuer, C. Ehrhardt, H. Bakowsky, M.N. Kumar, V. Oberle, C.M. Lehr, et al., The influence of physicochemical parameters on the efficacy of non-viral DNA transfection complexes: a comparative study, J. Nanosci. Nanotechnol. 6 (9–10) (2006) 2776–2782.
[15] C. Kollmann, R.A. Bezemer, K.E. Fredfeldt, U.G. Schaarschmidt, C.J. Teirlinck, A test object for quality control of the instrument for doppler (duplex) ultrasonography, based on the draft IEC 61685 standard, Ultraschall. Med. 20 (6) (1999) 248–257.
[16] C.W. Francis, S. Totterman, Magnetic resonance imaging of deep vein thrombi correlates with response to thrombolytic therapy, J. Thromb. Haemost. 73 (3) (1995) 386–391.
[17] M. Loren, L.J. Garcia Frade, M.C. Torrado, J.L. Navarro, Thrombus age and tissue plasminogen activator mediated thrombolysis in rats, Thromb. Res. 56 (1) (1989) 67–75.
[18] A.V. Alexandrov, R. Mikulik, M. Ribo, V.K. Sharma, A.Y. Lao, G. Tsivgoulis, et al., A pilot randomized clinical safety study of sonothrombolysis augmentation with ultrasound-activated perflutren-lipid microspheres for acute ischemic stroke, Stroke 39 (5) (2008) 1464–1469.
[19] C.A. Molina, M. Ribo, M. Rubiera, J. Montaner, E. Santamarina, R. Delgado-Mederos, et al., Microbubble administration accelerates clot lysis during continuous 2-MHz ultrasound monitoring in stroke patients treated with intravenous tissue plasminogen activator, Stroke 37 (2) (2006) 425–429.
[20] J. Pagola, M. Ribo, J. Alvarez-Sabin, M. Lange, M. Rubiera, C.A. Molina, Timing of recanalization after microbubble-enhanced intravenous thrombolysis in basilar artery occlusion, Stroke 38 (11) (2007) 2931–2934.
[21] F. Perren, J. Loulidi, D. Poglia, T. Landis, R. Sztajzel, Microbubble potentiated transcranial duplex ultrasound enhances IV thrombolysis in acute stroke, J. Thromb. Thrombolysis 25 (2) (2008) 219–223.
[22] A.F. Prokop, A. Soltani, R.A. Roy, Cavitational mechanisms in ultrasound-accelerated fibrinolysis, Ultrasound. Med. Biol. 33 (6) (2007) 924–933.
[23] K. Mizushige, I. Kondo, K. Ohmori, K. Hirao, H. Matsuo, Enhancement of ultrasound-accelerated thrombolysis by echo contrast agents: dependence on microbubble structure, Ultrasound. Med. Biol. 25 (9) (1999) 1431–1437.
[24] W.C. Culp, T.R. Porter, J. Lowery, F. Xie, P.K. Roberson, L. Marky, Intracranial clot lysis with intravenous microbubbles and transcranial ultrasound in swine, Stroke 35 (10) (2004) 2407–2411.
[25] B. Krasovitski, E. Kimmel, Stability of an encapsulated bubble shell, Ultrasonics 44 (2) (2006) 216–220.
[26] M.A. Borden, G. Pu, G.J. Runner, M.L. Longo, Surface phase behavior and microstructure of lipid/PEG-emulsifier monolayer-coated microbubbles, Colloids Surf. B: Biointerfaces 35 (3–4) (2004) 209–223.
[27] D.H. Kim, M.J. Costello, P.B. Duncan, D. Needham, Mechanical properties and microstructure of polycrystalline phospholipid monolayer shells: novel solid microparticles, Langmuir 19 (20) (2003) 8455–8466.
[28] D.B. Tata, F. Dunn, Thermodynamic method of B/A analysis of liposome suspensions: prediction via single relaxation theory, J. Ultrasound Med. 11 (8) (1992) 441–447.
[29] A. Schroeder, Y. Avnir, S. Weisman, Y. Najajreh, A. Gabizon, Y. Talmon, et al., Controlling liposomal drug release with low frequency ultrasound: mechanism and feasibility, Langmuir 23 (7) (2007) 4019–4025.